# **PCT**





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(54) Title: DNA DIAGNOSTICS BASED ON MASS SPECTROMETRY

#### (57) Abstract

Fast and highly accurate mass spectrometry-based processes for detecting a particular nucleic acid sequence in a biological sample are provided. Depending on the sequence to be detected, the processes can be used, for example, to diagnose a genetic disease or chromosomal abnormality; a predisposition to a disease or condition, infection by a pathogenic organism, or for determining identity or heredity.

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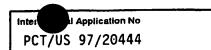
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(	see page 15, line 34 - page 18, line 10; examples 5,8	19-34, 82,83				
(	see example 8	42 47				
	see page 16, line 4; figures 6A,8 page 36, ln 33	47 48,49, 80,81				
	see page 26, line 7 see page 16 - page 18, line 10; figures	50-64, 68-70				
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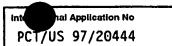
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....ernational application No.

PCT/US 97/20444

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:  SEE ANNEXES
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.

### FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

It should be further noted that "claims 82-83" as designated herein refer to two claims 82 and two claims 83 which were filed as follows, claims 82, 83 followed by a second claim 82 and a second claim 83)

1. Claims 1-18, partially 82-83:

A method for determining the sequence of a target nucleic acid involving the generation of base specifically terminated fragments.

2. Claims 19-34, partially 82-83:

A mothed for detecting a target nucleic acid present in a biological sample based on a nested polymerase chain amplification reaction.

3. Craim 35 partially (in that it relates to the detection or neoplasia/malignancies by detecting telomerase), craims 36 and 37, and partially 32-83:

An assay for the detection of neoplasia/malignancies based on telemerase specific extension of a substrate primer and a subsequent amplification of the telemerase specific extension product by PCR.

4. Claim 35 partially (in that it relates to the detection of neoplasia/malignancies by detecting mutation of a proto-oncogene), claims 38 and 39, and partially claims 82-80:

An assay for the detection of neoplasia involving mutation analysis of mutant or wild-type alleles by primer extension reaction by a Sanger type sequencing protocol.

### FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. Claim 35 partially (in that it relates to the detection of neoplasia/malignancies by detecting expression of a tumour-specific gene in a specific tissue type), claims 40 and 41, and partially claims 82-83:

An amplification based assay for the expression of the tyrosine hydroxylase gene in bone marrow cells as indicative or a neuropiastoma.

6. Claim 42, partially claims 82-83:

A method for directly detecting double stranded nucleic acid using Maldi-TOF mass spectrometry.

7. Claims 43-45, partially claims 82-63:

A method for comparing ONA relatedness by amplification or microsatellite DNA rapeat sequences.

Laim (c) partially claims 82-80:

A method for detecting mutations based on target amplification using a primer that introduces a unique endonuclease restriction site into amplified target and a combination of a Sanger sequencing protocol and endonuclease digestion.

9. Claim 47, partially claims 82-83:

a method for the amplification and detection of a nucleic acid based on the synthesis of RNA using a primer containing a RNA polymerase promoter sequence.

13. Claims 48, 49, 80 and 81, partially 82-83:

### FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Primers per se for mass spectrometry comprising a mass meaifying moiety.

11. Claims 50-64, partially 68-70, partially 73-79, partially claims 82-83:

Method for detecting a target nucleic acid sequence involving hybridisation to a detector oligonucleotide.

12. Claims 65-67, partially 68-70, 71-72, partially 75- $\frac{1}{1}$ 9, partially claims 82-83:

Methods for determining a nucleic acid sequence involving examplesse digestion.

#### 13. Claims 84-94:

Photorabile linkers per se for use in immobilisation of nucleic acids to solid supports.

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